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Rise of subgen. *Rhoicosphenula* Lange-Bertalot to the genus level, and description of a new *Gomphosphenia s.s.* species from Puerto Rico

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Rise of subgen. *Rhoicosphenula* Lange-Bert. to the genus level, and description of a new *Gomphosphenia s.s.* species from Puerto Rico

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ABSTRACT
The subgenus *Rhoicosphenula* Lange-Bert. has a unique character combination that differentiates it from *Gomphosphenia* subgen. *Gomphosphenia* Lange-Bert. In particular, *Rhoicosphenula* has striae/areola features that are similar to *Gomphosphenia*, but is differentiated from that genus by also having pseudosepta present on both poles of the valves, copulae with septa at one of the poles, and frustules almost rectangular in girdle view. Together, this character combination strongly supports it as an independent genus following modern diatom-taxonomy criteria. Moreover, the only species of *Rhoicosphenula* known so far was

collected from a peculiar acidic and dystrophic habitat, whilst most *Gomphosphenia s.s.* species colonize medium-conductivity, alkaline inland waters. On the basis of LM and SEM studies, we also propose a species of *Gomphosphenia* (*G. patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov.) as new to science. The new species is mainly defined by its valve outline and dimensions, length to breadth ratio, striation density, and geographical distribution. It was found in several samples of two streams in Puerto Rico, sometimes with high relative abundance. *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov. was much more abundant in the stream with lower nutrient (in particular P) concentrations, and higher ecological integrity. The new species seems to prefer moderately alkaline freshwater habitats with medium-high conductivity and oligo- to mesotrophic conditions, with moderate tolerance to N enrichment. Also noteworthy is the occurrence of the new species in streams with above-average magnesium concentrations (due to the local geology). This Neotropical, warm-water species might be a potential indicator of global warming effects.

KEY WORDS
Gomphosphenia,
Rhoicosphenula,
diatoms,
Puerto Rico,
tropical streams,
new status,
new combination,
new species.

RÉSUMÉ

Élévation du sous-genre Rhoicosphenula *Lange-Bert. au niveau de genre et description d'une nouvelle espèce de* Gomphosphenia s.s. *de Porto Rico.*

Le sous-genre *Rhoicosphenula* Lange-Bert. possède une combinaison unique de caractères qui le différencie du sous-genre *Gomphosphenia* Lange-Bert. Notamment, *Rhoicosphenula* a des traits de stries/aréoles qui sont similaires à ceux de *Gomphosphenia*, mais se différencie de ce genre par la présence de pseudosepta sur les deux pôles des valves, de copules avec des septa à l'un des pôles, et de frustules presque rectangulaires vu depuis la ceinture. Ensemble, cette combinaison de caractères soutient fortement le genre comme un genre indépendant selon les critères modernes de la taxonomie des diatomées. De plus, la seule espèce de *Rhoicosphenula* connue jusqu'à présent a été collectée dans un habitat acide et dystrophique particulier, alors que la plupart des espèces de *Gomphosphenia s.s.* colonisent des eaux intérieures alcalines et de conductivité moyenne. Sur la base des études de microscopie optique et microscopie électronique à balayage, nous proposons également une espèce de *Gomphosphenia* (*G. patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov.) comme nouvelle pour la science. La nouvelle espèce est principalement définie par le contour et les dimensions de ses valves, le rapport longueur/largeur, la densité des striations et la distribution géographique. Elle a été trouvée dans plusieurs échantillons de deux ruisseaux à Porto Rico, parfois avec une abondance élevée. *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov. était beaucoup plus abondant dans le ruisseau avec des concentrations de nutriments plus faibles (en particulier P), et un meilleur état écologique. La nouvelle espèce semble préférer les habitats d'eau douce modérément alcalins avec une conductivité moyennement élevée et des conditions oligo- à mésotrophes, avec une tolérance modérée à l'enrichissement en azote. Il convient également de noter sa présence dans des cours d'eau présentant des concentrations de magnésium supérieures à la moyenne (en raison de la géologie locale). Cette espèce néotropicale d'eau chaude pourrait être un indicateur potentiel des effets du réchauffement climatique.

MOTS CLÉS
Gomphosphenia,
Rhoicosphenula,
diatomés,
Porto Rico,
ruisseaux tropicaux,
statut nouveau,
combinaison nouvelle,
espèce nouvelle.

INTRODUCTION

The diatom genus *Gomphosphenia sensu lato* Lange-Bert. (family Rhoicospheniaceae) was established in 1995, and included two subgenera: *Gomphosphenia* and *Rhoicosphenula* (Lange-Bertalot 1995). *Gomphosphenia lingulatiformis* (Lange-Bertalot & E. Reichardt) Lange-Bert. was designated as the generitype of the genus *Gomphosphenia sensu lato*. It was at the same time the subgeneritype of the subgenus *Gomphosphenia* whilst *Gomphosphenia paradoxa* Lange-Bert., of Neotropical origin, was selected as the subgeneritype of subgen. *Rhoicosphenula*. Overall, the key morphological differences on which the two subgenera were separated taxonomically were the presence of distinctive pseudosepta at both valve poles and septa at one pole of the copulae in *Rhoicosphenula*, and the almost rectangular girdle view (vs pseudosepta and septa completely absent, and girdle view typically wedge-shaped in *Gomphosphenia*).

The genus *Gomphosphenia* sensu lato was separated from the genus *Gomphonema* Ehrenberg based on unique character combinations, where the main morphotaxonomic features of the former do not fit within the restricted taxonomic concept of the latter (Round *et al.* 1990): 1) absence of external areolar occlusions found in most species of *Gomphonema*; 2) terminal raphe fissures usually terminating on the valve face; 3) internal proximal raphe ends usually T-shaped (with exceptions in a few species); 4) absence of apical pore fields and stigma(ta); and 5) striae usually composed of single areolae (foramina), with exceptions in a few species in which striae are made up of a few areolae (Lange-Bertalot 1995 and the references therein). A recent cladistic study conducted by Majewska *et al.* (2015) on some gomphonemoid marine and freshwater taxa, including the genera *Gomphosphenia*, *Rhoicosphenia*, and *Gomphonema*, confirmed the identity of the genus *Gomphosphenia* within the family Rhoicospheniaceae. Additionally, Ress *et al.* (2016) and Kociolek *et al.* (2019) emphasized that the gomphonemoid outline of *Gomphosphenia* is the result of convergence, not homology, with other gomphonemoid lineages.

Over the past 25 years, several interesting species of *Gomphosphenia* have been discovered and reported as species new to science from diverse and geographically distant, mainly freshwater, habitats worldwide, e.g. *G. fontinalis* Lange-Bert., Ector & Werum from freshwater springs in Germany (Werum & Lange-Bertalot 2004), *G. stoermeri* Kociolek & E.W. Thomas as epilithic species from Cataract Falls in the Great Smoky Mountains National Park, United States (Thomas *et al.* 2009), *G. tenuis* Levkov & D.M. Williams from St. Naum springs and Lake Ohrid, Macedonia (Levkov & Williams 2011), *G. americana* Kociolek & E.Thomas and *G. indistincta* Kociolek & E. Thomas from Blanchard Lake in Utah, United States (Kociolek *et al.* 2014), *G. praegnans* J.A. Ress, E.W. Thomas & Kociolek from Rocky Mountain Front Range streams, Colorado, United States (Ress *et al.* 2016), *G. ryukyuensis* A. Tuji & Ohtsuka from Yamato-ga cave, Miyako Island, Japan (Tuji 2016), and *G. biwaensis* Ohtsuka & Nakai as epiphytic species from the freshwater Biwa Lake, Japan (Ohtsuka *et al.* 2018). To date, 19 different species and infraspecific taxa belonging to the genus *Gomphosphenia* s.l. have been described, and, of these, 17 taxa have been accepted taxonomically (Guiry & Guiry 2021 and the references therein). Ecologically, species of *Gomphosphenia* have been documented to be able to propagate in a wide niche, from oligotrophic to eutrophic conditions (e.g., Thomas *et al.* 2009; Van de Vijver *et al.* 2012; Noga *et al.* 2016; Udovič *et al.* 2018).

Puerto Rico is an unincorporated territory of the United States located in the northeast Caribbean Sea, and approximately 1600 km southeast of Miami, Florida. It has a total area of 9104 km^2. This tropical island has an average temperature of 28°C throughout the year. Diversification of diatom flora of two Puerto Rico streams has been regularly surveyed and extensively studied as part of the United States National Ecological Observatory Network (NEON) program. Among the fruitful findings recently published is, for instance, the discovery of the freshwater diatom species *Adlafia neoniana* Cantonati (Ciugulea *et al.* 2019) and *Grunowia portoricensis* Kociolek, Danz, Swenson, Thirouin, Williams & Borsa (Kociolek *et al.* 2020) from Puerto Rico forest streams, supporting the partial novelty and potential interest of the diatom flora in these tropical aquatic environments (Cantonati *et al.* 2020). In this paper, we raise the subgenus *Rhoicosphenula*, one of the two subgenera of the genus *Gomphosphenia* sensu lato, to the genus level. We also describe an epilithic *Gomphosphenia* taxon from two streams in Puerto Rico as species new to science. Key morphotaxonomic features and ecological preferences of this new freshwater diatom species are presented, as well as a comparison with the morphologically most similar species.

MATERIAL AND METHODS

Study Area

The *Gomphosphenia* specimens investigated in this study were sampled, as part of the NEON program, from two Neotropical streams located in southwestern Puerto Rico (compare Ciugulea *et al.* 2019): Rio Cupeyes (18°06'48.65"N, 66°59'12.30"W, elevation: 166 m a.s.l.), and Rio Guilarte (18°10'26.77"N, 66°47'54.73"W, elevation: 548 m a.s.l.). Overall, the two streams have low to medium discharge (up to 230 L.s^{-1}), warm, weakly-alkaline to alkaline waters (average pH: 7.76 and 8.08, respectively), and medium conductivity (up to 400 µS.cm^{-1}) (Fig. 1).

The Rio Cupeyes study site (NEON code: CUPE; Academy of Natural Sciences site code: NFS008, site_id: 207563) is in the Maricao State Forest and is a NEON aquatic core field site. The site host is the Commonwealth of Puerto Rico. The lithology is serpentinite rocks (Cretaceous). The area is made up of old and second-growth tropical, moist and wet forest. It is considered a good example of an increasingly rare intact Puerto Rican stream (Ciugulea *et al.* 2019). The average magnesium concentration was relatively high with a value of 31.53 mg.L^{-1}. Calcium concentration was low (average: 4.09 mg.L^{-1}). For nutrients, average total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were 352 and 2 µg.L^{-1}, respectively (Fig. 1).

The Rio Guilarte study site (NEON code: GUIL; Academy of Natural Sciences site code: NFS009, site_id: 207564) is a relocatable (= may be re-deployed periodically) NEON aquatic field site. The site host is the University of Puerto Rico. The lithology is characterized by the Anon formation: interbedded andesite/volcanic breccia, rhyodacite, and sandstone/siltstone of the Upper Eocene. Calcium was the most prevailing cation with an average concentration value of 25.55 mg.L^{-1}, while average magnesium was 5.50 mg.L^{-1}. Average TDN and TDP were 583 and 19 µg.L^{-1}, respectively (Ciugulea *et al.* 2019) (Fig. 1).

Diatom sampling, preparation, and identification

The samples on which this study is based were taken at the Cupeyes and Guilarte sites in the context of the NEON program sponsored by the National Science Foundation (United States) and operated under cooperative agreement by Battelle Memorial Institute, United States. All sample information and diatom counts can be downloaded from the NEON data portal: http://data.neonscience.org/home. 82 epilithon samples from the CUPE (37 samples) and GUIL (45 samples) streams were studied. On each sampling campaign, a primary (5 composite samples) and, usually but not always, a secondary (3 samples) habitat was sampled. Primary habitat was "riffle" in both rivers. Secondary habitat was "run" in CUPE and "pool" in GUIL. The sampling stations are long stream reaches (1 km); riffle (and run and pool) samples were taken along this stretch. Three cobbles for each composite sample were collected randomly. A halved 35-mm slide cassette was used as template to demarcate sampling area. Periphyton was scraped from the cobbles with a brush (Parker 2018).

Diatom samples were digested following the NAWQA protocols (Charles *et al.* 2002). After repeated washing and centrifugation, the cleaned diatom material was mounted in Naphrax® (refractive index of 1.74). At least 600 valves were characterized and counted using a Zeiss Ser. N. 800392 (Zeiss, Jena, Germany) and ×1000 magnification (ANSP Drexel

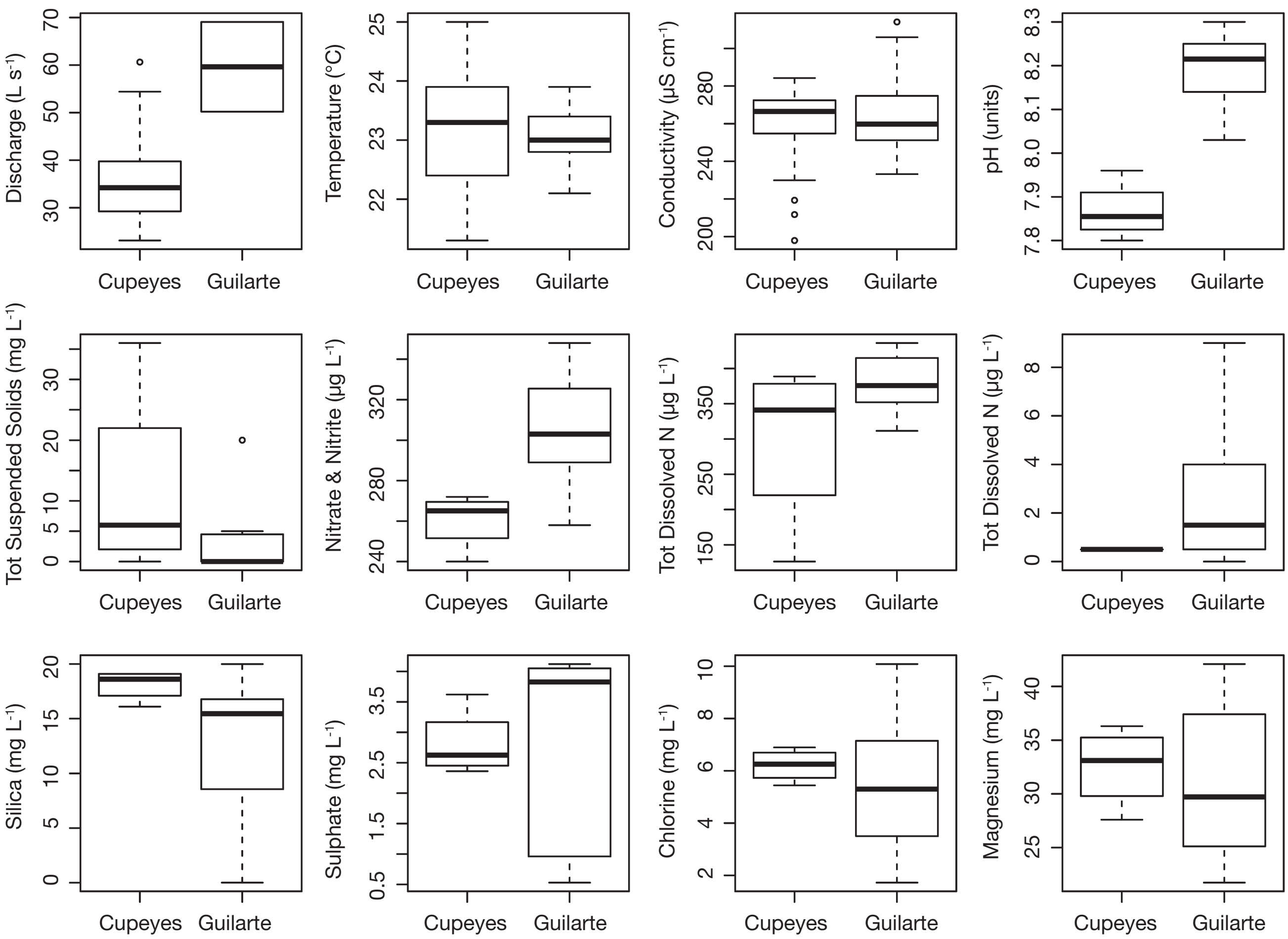

FIG. 1. — Box-and-whisker plots for selected physical and chemical variables for the two study streams.

PCER Procedure No. P-13-39 Revision 2 02/17). High-quality Nomarski (DIC) LM micrographs were taken using a Zeiss Akioskop 50 microscope with Zeiss Plan-Apochromat 100×/1.40 Oil objective lens and Nikon Digital Sight DS Fi1 digital camera.

Materials (permanent slides, cleaned material, and aliquots of the original samples), including the holotype slide, are housed at the ANSP Diatom Herbarium (ANSP). Isotype slides and aliquots of prepared materials from the same locality and substratum were deposited in the NEON Biorepository in Arizona, the Diatom Collection of the Natural History Museum (BM) of London (United Kingdom), the Diatom Collection of the MUSE - Museo delle Scienze (TR), Trento, Italy, and the diatom collection of the Phycology Unit (No. 341), the Botany Department, Faculty of Science, Ain Shams University, Cairo (Egypt) curated by Abdullah A. Saber. Measurements on at least 30 different specimens representative of the size-diminution series were made to obtain biometric data on the key morphological and fine ultrastructural features, and also to compare them with other allied taxa. Scanning electron microscopy (SEM) was primarily conducted using a Hitachi S-4500 (Hitachi Ltd., Tokyo, Japan) at high vacuum on gold-coated stubs. To confirm the absence of septa and pseudosepta and find more internal views, further SEM observations were done at the MUSE - Museo delle Scienze using a LEO XVP (Carl Zeiss SMT Ltd., Cambridge, United Kingdom) at high vacuum. Since most specimens were closed frustules in girdle view, and the few valvar views were almost all external views, we carried out SEM observations also on sonicated material (9 min, 35 KHz, in a Bandelin Sonorex super, BANDELIN electronic GmbH & Co. KG, Berlin, Germany). Terminology for the valve morphology was based on the relevant literature of Round *et al.* (1990) and Lange-Bertalot (1995).

Since no new material of the apparently very rare species *Gomphosphenia* (*Rhoicosphenula*) *paradoxa* became available since one of us wrote the seminal paper in German on the genus *Gomphosphenia* (Lange-Bertalot 1995), the data and illustrations used for *Rhoicosphenula* in this paper were taken from Lange-Bertalot (1995).

STATISTICAL ANALYSES

The ecological preferences of *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov., were studied assessing three factors: river (Cupeyes, Guilarte; df

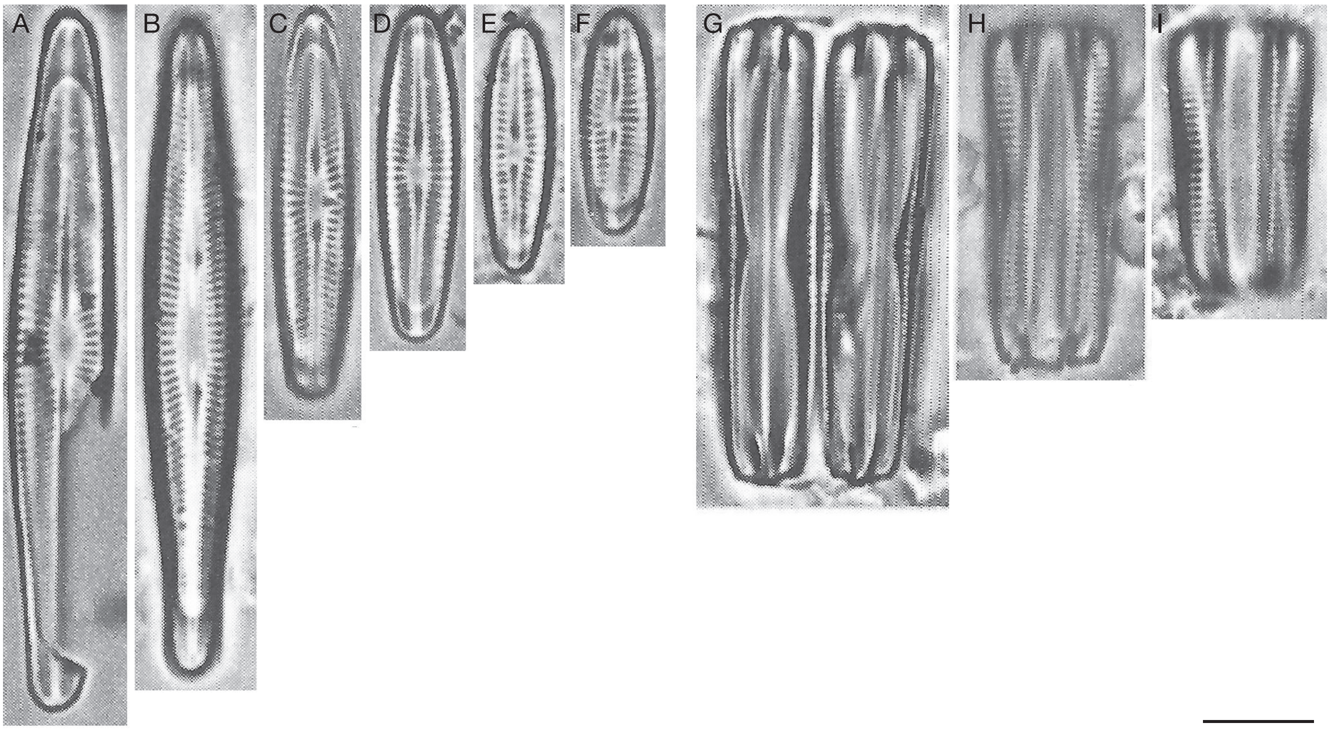

Fig. 2. — LM micrographs of the generitype species (*R. paradoxa*) of *Rhoicosphenula* Lange-Bert., Kociolek, A.A.Saber & Cantonati, stat. nov., depicting the valve size-diminution series in valve (**A**-**F**) and girdle view (**G**-**I**); G undergoing division process) of the holotype specimens. Note the pseudosepta at the head- and footpole, the proximal raphe pores which are well separated and broadened (**A**-**F**) whilst the internal endings are closer to each other (see e.g., bright contours in B). Scale bar: 10 µm. From Lange-Bertalot (1995) mod.

= 1), habitat (riffle, run, pool; df = 2), and season (autumn, winter, and summer; df = 2). We performed Kruskal–Wallis tests, a non-parametric method, which does not assume a normal distribution of the residuals, and is often used in the case of different sample sizes (Sokal & Rohlf 1995). A three-way analysis of variance (ANOVA) was used to test the significance of the interactions among the individual factors (rivers, seasons, habitats). All the analyses were carried out using R (R Core Team 2018).

RESULTS

Description of *Rhoicosphenula* raised to the genus level

Family Rhoicospheniaceae Chen & Zhu

Rhoicosphenula
Lange-Bert., Kociolek, A.A.Saber & Cantonati, stat. nov.
(Figs 2, 3)

Rhoicosphenula Lange-Bert. *Nova Hedwigia* 60: 244 (1995).

Cells solitary, not forming colonies or aggregates, growing as epiphytes on small mucilaginous stalks. Frustules almost rectangular in girdle view, sometimes appearing slightly broadly wedge-shaped (Fig. 2G-I). Pseudosepta present on both poles of the valves. Raphe branches straight, with large, well-separated proximal ends (Fig. 2A-F), and with T-shaped distal fissures (Fig. 3A). Internal fissures extend onto the central nodule, with short and very faintly bent proximal ends (Fig. 3C, H). Foramina of the areolae simple, more or less slit-shaped, forming two systems, one on the valve face and one on the mantle, respectively, separated by a hyaline mantle margin (Fig. 3A, B). Areolae are covered internally by flat hymenes (similar to those of *Brachysira*), developing from the sternum to the mantle without any visible interruption (Fig. 3F, G). The cingulum composed of six unilaterally open bands (Fig. 3B), including the valvocopula and first copula (each with a septum at one of the poles), as well as the four further copulae (Fig. 3C, D).

Registration. — http://phycobank.org/102718

New combinations within Rhoicosphenula *stat. nov.:*

Rhoicosphenula paradoxa (Lange-Bert.) Lange-Bert., Kociolek, A.A.Saber & Cantonati, comb. nov.
(Figs 2, 3)

Gomphosphenia paradoxa Lange-Bert., *Nova Hedwigia*, 246, pls I-III (1995).

Registration. — http://phycobank.org/102719

Distribution
This is the type species of this still monotypic genus. It was collected from the Neotropical, black-water River Tec in Venezuela, at 1200 m a.s.l., in the surroundings of the Roraima Tepui, a table-top mountain formed by remains of a large sandstone plateau that once covered the granite bedrock. Besides *R. paradoxa* comb. nov., the type material includes several representatives of the genera *Eunotia*, *Actinella*, and

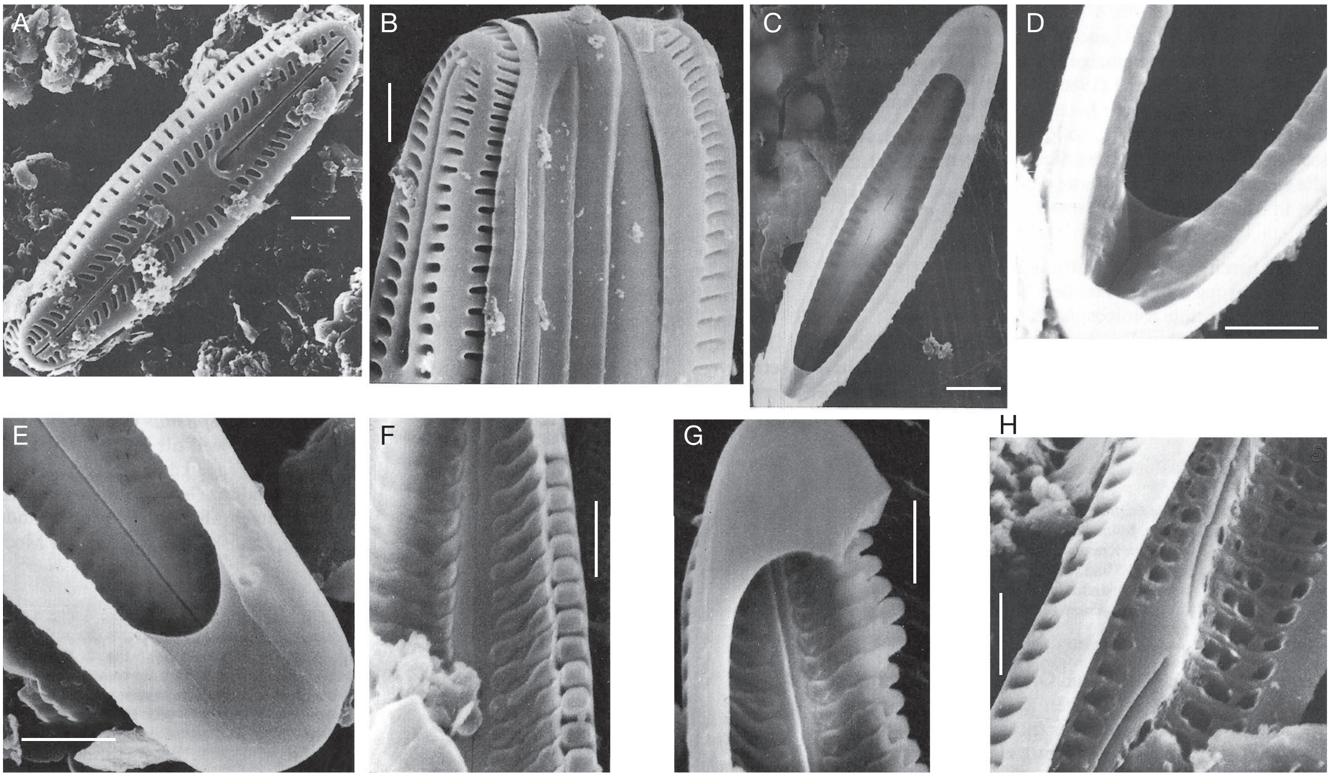

Fig. 3. — SEM micrographs of the generitype species (*R. paradoxa*) of *Rhoicosphenula* Lange-Bert., Kociolek, A.A.Saber & Cantonati, stat. nov. External views (A, B); internal views (C-H): **A**, complete frustule (note the slit-like areole on valve face and mantle); **B**, hypovalve (left) with footpole immersed in the six girdle bands, which appear totally hyaline; they are alternately open and closed at each pole; **C**, valve with apposed valvocopula and a further copula; **D**, headpole; the valvocopula forms a septum; the valve pseudoseptum is thus mostly covered, only a triangle remains visible; **E**, valve footpole with pseudoseptum and open valvocopula (no further copula); **F**, exposed fracture of the right valve margin: the transapical ribs and the alveoli covered by hymenes become visible; **G**, exposed fracture at the pseudoseptum; **H**, the corroded hymenes allow a view of the areolae which are more numerous and differently shaped than the slit-like external foramina. Scale bars: A, C, 2 µm; B, D-H, 1 µm. From Lange-Bertalot (1995) mod.

Brachysira, as well as other species indicating high humic-acids content.

Class BACILLARIOPHYCEAE Haeckel
Order CYMBELLALES D.G.Mann
Family Rhoicospheniaceae J.Chen & H.Zhu
Genus *Gomphosphenia* Lange-Bert.

Gomphosphenia patrickiana
Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov.
(Figs 4-6)

Type material. — **Unites States**. Puerto Rico, Cupeyes Stream, National Forest, south-western part of the Island of Puerto Rico, lithology: serpentinites (metamorphic rocks), (18°06'48.65"N, 66°59'12.30"W, 166 m a.s.l.), 3.II.2016 (holo-, ANSP, slide NEON00303 b, partly shown here in Fig. 4; iso-, BM, slide BM 81900; iso-: TR, slide cLIM004 DIAT 3904; NEON Biorepository, Arizona State University's Natural History Collection in Tempe, AZ, slide NEON00303 a; diatom collection of the Botany Department, Faculty of Science, Ain Shams University, Egypt, slide PBA–DIAT 2001).

Registration. — http://phycobank.org/102720

Etymology. — The specific epithet "*patrickiana*" is named in honor of the United States phycologist and limnologist specializing in diatoms and hydrobiology, Ruth M. Patrick (1907-2013). She developed innovative ways to assess the quality of freshwater ecosystems, in particular using diatoms, authored >200 scientific papers, and established numerous research facilities, in particular the Phycology Section at the Academy of Natural Sciences of Philadelphia (ANSP, part of Drexel University since 2011). We consider this last achievement notable in the international museological context, as a bright example of a part of the research division (ANSP's Patrick Center for Environmental Research) of a science museum that obtains most of its funding from tenders, consulting, and ecological assessment projects, many carried out at a nation-wide scale.

Morphology

Light microscopy (Fig. 4M-U)

Frustules wedge-shaped in girdle view (Fig. 4M-U). Valves linear-clavate in larger specimens to clavate in smaller ones, slightly broader at the central area in the larger specimens, the headpoles rounded whilst the footpoles are narrowly rounded (Fig. 4A-L), 8.0-17.5 µm long, 2.5-4.0 µm wide, L/W ratio: 3.4-4.8. Axial area lanceolate to moderately narrower in the smaller specimens, in general narrow at the poles and gradually widening towards the central part. Central area ± rhombic, mostly with 2-4 shortened striae, and in some specimens transversely expanded to the valve margin on one side forming a unilateral fascia (Fig. 4C). The raphe straight, filiform with distinct proximal endings. Striae radiate throughout the valve, becoming almost parallel near the head- and footpole, 23-27 in 10 µm.

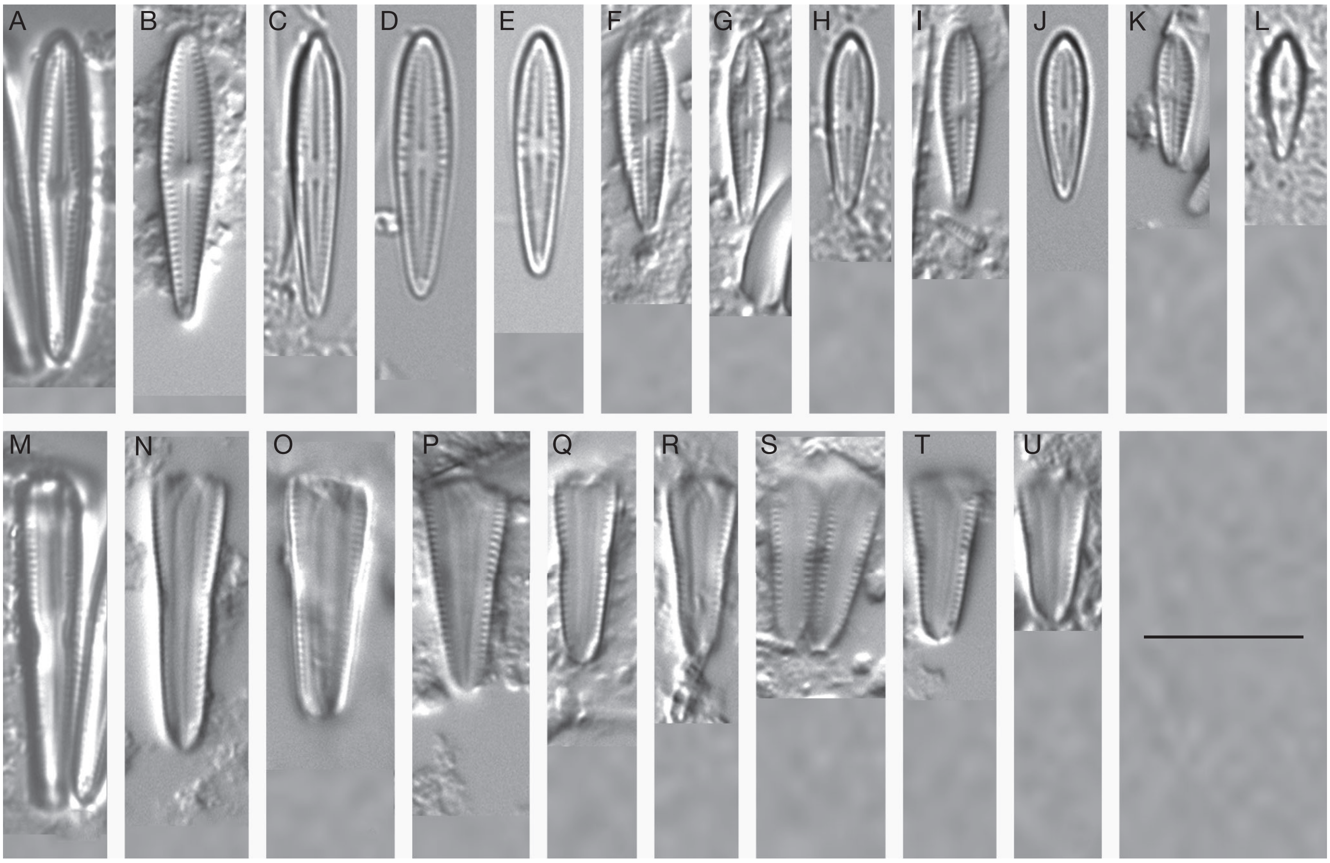

Fig. 4. — **A**-**U**, Light micrographs of *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov., depicting the valve size-diminution series and girdle view of the holotype specimens. Scale bar: 10 µm.

Scanning electron microscopy (Figs 5; 6)

The valve face is flat, without stigma. Striae on the valve exterior composed of single radiate slit-like areolae 'foramina', becoming almost round poroids near the poles, particularly the footpole (Fig. 5A, B). Central area in some specimens with a broad unilateral fascia (Fig. 5A), and slightly broader in the larger specimens (Fig. 5B). Raphe straight, filiform with distinctly dilated proximal raphe endings, and distal raphe fissures terminating on the valve face, not extending into the valve mantle, with teardrop-shaped pores (Fig. 5A, B). The valve mantle bears a single row of areolae which are slit-like at the upper valve half and the headpoles but rounded in shape near the mid-valve and towards the footpole (Fig. 6C, D). Internally, septa and pseudosepta at both poles are absent (Fig. 5C; 6F, G). Internal foramina have almost the same size as the external areolae, and are occluded by hymenes (Fig. 5C, D). Central nodule is slightly raised (Fig. 5D). Internal proximal raphe endings are T-shaped (Fig. 5C, D), while the internal terminal raphe fissures terminate in relatively large helictoglossae (Fig. 6A, B). In girdle view, frustules typically wedge-shaped, with cingulum composed of seven open bands, each bearing one row of small round pores. Apical pore fields absent (Fig. 6C, D, E).

Ecology and co-occurring diatom species

So far, *G. patrickiana* has been observed only as a Neotropical epilithic species in the two rivers Rio Cupeyes and Rio Guilarte, located in southwest Puerto Rico. It was reported in 27 out of the 37 samples investigated from Rio Cupeyes, and in 21 out of the 45 samples from Rio Guilarte. Maximum relative abundance was distinctly higher in Rio Cupeyes (43%) compared to Rio Guilarte (3%). The Rio Cupeyes is, in general, characterized by a water quality much better than that of the agriculture-impacted Rio Guilarte stream, in terms of average TDN and TDP values (Fig. 1).

Only epilithon samples were available for this study but they were collected from different habitats and stream reaches (within the same station). Table 1 shows the distribution of the species (using per cent relative abundance) with respect to stream and microhabitat, showing that it was clearly more abundant and frequent in the Rio Cupeyes (Kruskal-Wallis chi-squared "streams" = 14.37, d.f. = 1, p = 0.0001). Similar results were obtained for microhabitats (Kruskal-Wallis chi-squared "habitats" = 11.01, d.f. = 2, p = 0.004) and seasons (Kruskal-Wallis chi-squared "seasons" = 10.92, d.f. = 2, p = 0.004). However, a three-way ANOVA showed that the interaction among these three factors is significant (factor =

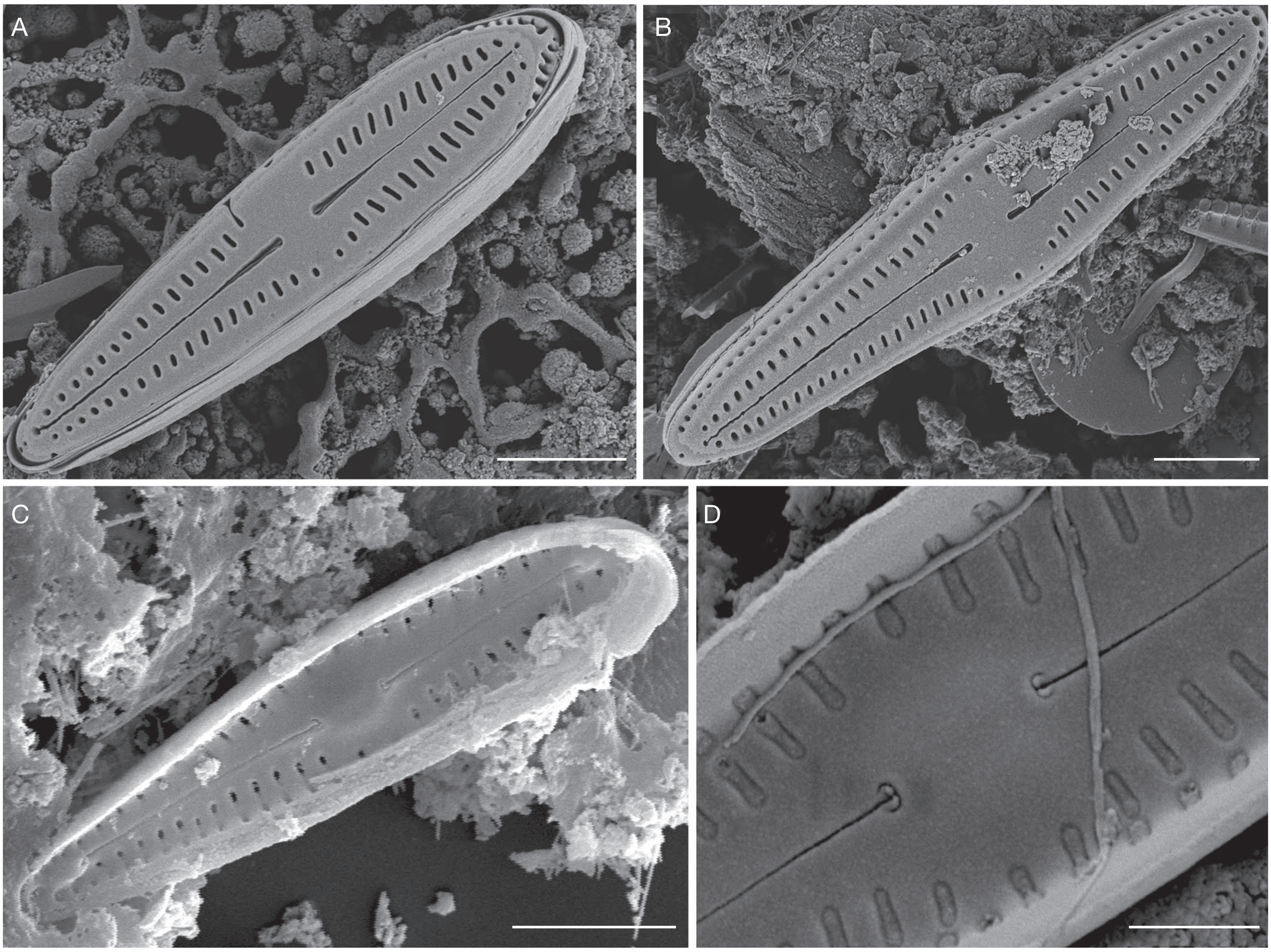

FIG. 5. — SEM micrographs of *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov.: **A**, **B**, external views of whole valves showing the striation pattern; central area slightly broader in larger specimens (**B**); unilateral fascia might be present (**A**); **C**, internal view of a whole theca + parts of the valvocopula; pseudosepta and septa absent; **D**, close-up view on the internal valve mid-section, depicting the slightly elevated central nodule and the T-shaped proximal raphe endings. Scale bars: A-C, 3 µm; D, 1 µm.

TABLE 1. — Distribution of *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov., in the two study streams and habitats using per cent relative abundance. Abbreviations: s.d., standard deviation; s.e., standard error; c.i., confidence interval.

stream	habitat	N	mean %	s.d.	s.e.	c.i.
Cupeyes	riffle	25	2.8	7.59	1.52	3.13
Cupeyes	run	12	8.0	14.23	4.11	9.04
Guilarte	pool	15	0.3	0.38	0.10	0.21
Guilarte	riffle	30	0.3	0.67	0.12	0.25

river × season, d.f. = 2, F = 8.20, p = 0.001; factor = season x habitat, d.f. = 4, F = 3.95, p = 0.006).

The predominant diatom species (relative abundance > 5%) of the most common genera at the type locality (Rio Cupeyes) during the whole period of study were: *Gogorevia constricta* (Torka) Kulikovskiy & Kociolek, *Achnanthidium jackii* Rabenhorst, *Adlafia neoniana* Cantonati, *Denticula occidentalis Østrup*, *Gomphonema neotropicum* N.Abarca & D.Mora, *Nitzschia paleacea* (Grunow) Grunow, *Sellaphora saugerresii* (Desmazières) C.E. Wetzel & D.G. Mann, and *Ulnaria lanceolata* (Kützing) Compère. Predominant species (rel. ab. > 5%) at the Rio Guilarte were: *Achnanthidium eutrophilum* (Lange-Bert.) Lange-Bert., *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow, *C. placentula* var. *lineata* (Ehrenberg) Van Heurck, *Craticula subminuscula* (Manguin) C.E. Wetzel & Ector, and *Cymbella turgidula* Grunow, *Gomphonema kobayasii* Kociolek & J.C. Kingston, *Halamphora veneta* (Kützing) Levkov, *Nitzschia* cf. *palea* (Kützing) W.Smith, *Sellaphora nigri* (De Notaris) Wetzel & Ector, *Ulnaria monodii* (Guermeur) Cantonati & Lange-Bert., and *U. ramesii* (Héribaud) T. Ohtsuka.

DISCUSSION

The original description of *Rhoicosphenula* as one of the two subgenera of the genus *Gomphosphenia* (Lange-Bertalot 1995) reflected a widespread conservative attitude in dia-

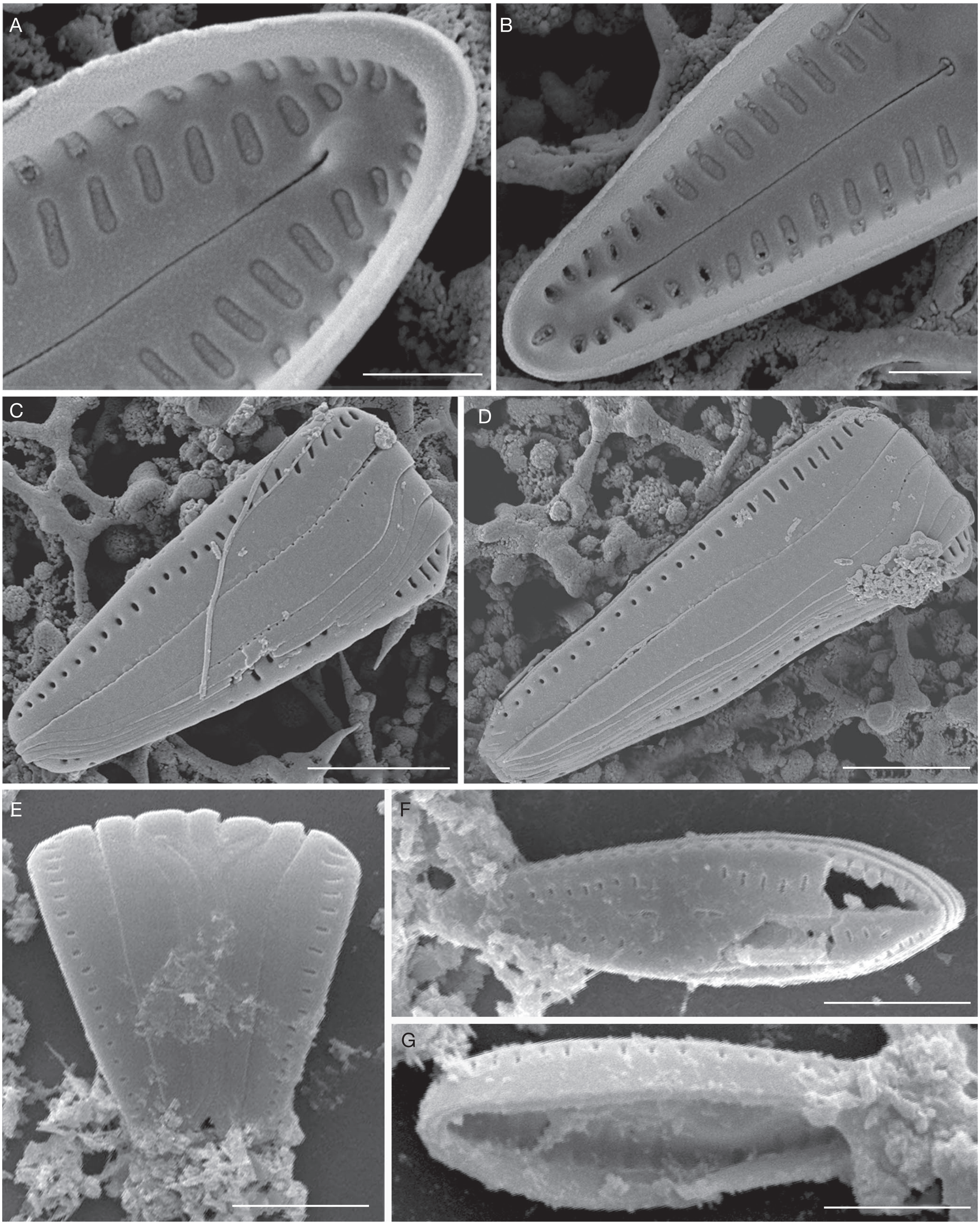

Fig. 6. — SEM micrographs of *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov.: **A**, **B**; close-up views of the internal pole regions of the valve showing relatively large helictoglossae; **C**-**E**, wedge-shaped girdle view of the frustules showing details of the valve mantle and cingulum bands; apical pore fields absent. **F**, **G**, external, with window due to fracture (F), and internal (G) view of a whole theca + parts of the valvocopula; pseudosepta and septa absent. Scale bars: A, B, 1 µm; C-G, 3 µm.

Table 2. — Comparison of key features distinguishing the genera *Gomphosphenia sensu stricto* and *Rhoicosphenula* as defined in this study.

	Gomphosphenia sensu stricto	***Rhoicosphenula* stat. nov.**
Main reference	this study	this study
Frustules' girdle view	typically wedge-shaped	almost rectangular
Septa	absent	conspicuous: copulae with septa at one of the poles
Pseudosepta	absent (faintly developed at the headpole in some species?)	distinctive at both valve poles
Terminal raphe fissures (external)	teardrop-shaped pores	T-shaped
Proximal raphe fissures (internal)	usually T-shaped	short and very faintly bent
Habitat	medium-conductivity, alkaline inland waters	acidic and dystrophic

tom taxonomy at that time, that kept the splitting and the number of new taxa as low as possible for the sake of the simplicity of the overall diatom taxonomic system. However, the combination of characters (Table 2) that identifies *Rhoicosphenula* and separates it from *Gomphosphenia* (i.e., pseudosepta present on both poles of the valves, copulae with septa at one of the poles, and frustules almost rectangular in girdle view) is so peculiar and straightforward that there would be no doubt in recognizing *Rhoicosphenula* as a separate genus according to current diatom taxonomy standards. A further support of this separation at the genus level comes from the ecological preferences of the members of the two genera: the only known species of *Rhoicosphenula* (*R. paradoxa* comb. nov.) was collected from a very peculiar acidic and dystrophic habitat whilst most *Gomphosphenia* species colonize medium conductivity inland waters with no or negligible preference for humic acids (Table 2).

The distinctive morphological features of *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov., typically coincide with the protologue of the genus *Gomphosphenia* subgenus *Gomphosphenia*, i.e., frustules most often club-shaped with a wedge-shaped girdle view, the raphe straight and filiform with terminal fissures not extending into the valve mantle, the internal proximal raphe endings T-shaped, and, eventually, the complete absence of stigma, pseudosepta, septa, and apical pore fields (Figs 4-6; Table 3). *G. patrickiana* differs from other morphologically similar taxa by having the following unique character combinations: the valve outline and dimensions (8.0-17.5 µm long and 2.5-4.0 µm wide), L/W ratio (3.4-4.8), striation density (23-27 in 10 µm), and biogeography (so far known only from Neotropical streams). None of the other established similar *Gomphosphenia* species share these combined morphological features. We consider *Gomphosphenia tackei* (Hustedt) Lange-Bert. the species bearing the greatest resemblance to the new species we are proposing here. In fact, a previous LM-based, floristic study on the diatoms from streams in Puerto Rico (Bryan 2001) reported *G. patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov. as *G. tackei*. Apart from the valve shape and width, *G. tackei* (figs 16-18 in Hustedt 1942, and fig. 165: 22-24 in Krammer & Lange-Bertalot 1986) readily differs from *G. patrickiana* by its longer valves (up to 26.0 µm vs 8.0-17.5 µm in *G. patrickiana*), relatively higher stria density (24-29 in 10 µm vs 23-27 in 10 µm in *G. patrickiana*), finely-punctate stria ultrastructure (*G. patrickiana* striae are mainly composed of single slit-like and round areolae), and the absence of a fascia (a unilateral fascia can sometimes be present in *G. patrickiana*) (Table 3). Another comparable diatom species is *G. holmquistii* (Foged) Lange-Bert. Though the larger specimens in both species share the presence of a broader central area, the valve dimensions, L/W ratio, and striae density are fairly different, and thus the two species can be easily distinguished from each other (Table 3). Noga *et al.* (2016) investigated *G. holmquistii* sampled from streams in southern Poland and found that the external proximal raphe ends were dilated into pores and slightly unilaterally deflected. This taxonomic feature is truly different in *G. patrickiana*, where the external proximal raphe endings are straight and distinctly expanded into pores (Fig. 5A, B). *G. lingulatiformis* (Lange-Bert. & E.Reichardt) Lange-Bert. differs from *G. patrickiana* by the following morphometric data: 1) the valve outline has undulate margins in the larger specimens, and headpoles are narrowly rounded to distinctly cuneate; 2) wider range of valve dimensions (12-50 µm long and 5.5-7.0 µm wide vs 8.0-17.5 µm long, 2.5-4.0 µm wide in *G. patrickiana*); 3) L/W ratio; 4) shapes of the wider axial and central areas; 5) much coarser stria density with the occurrence of additional 1-2 isolated areolae towards the axial area (this feature is absent in *G. patrickiana*); and 6) the distal raphe endings are unilaterally deflected (in *G. patrickiana* they terminate with teardrop-like pores) (Table 2). Smaller valves of *G. plenkoviciae* G. Udovič & Žutinić might be confused with *G. patrickiana* but there are still several morphological differences which never overlap. Apart from the clear discrepancies in their valve outlines, dimensions and L/W ratio (check Table 2 for these details), the valve mantle in *G. plenkoviciae* usually has 2-4 rows of rounded areolae, and in some specimens rarely single slit-like openings, compared to a single row of round and slit-like areolae in *G. patrickiana* on the valve mantle. Also, stria density in *G. plenkoviciae* is lower than that of *G. patrickiana*. Furthermore, *G. plenkoviciae* has striae composed of single round areolae at the mid-valve and slightly elongated slits towards the poles, and areolae are much longer internally than externally. Otherwise, in *G. patrickiana* there are single slit-like striae throughout the valve, which become round poroids towards the poles. Moreover, internal and external areolae are the same size (Fig. 5). All of these features differentiate *G. patrickiana*

TABLE 3. — Comparison of morphometric features and habitat between *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov., and morphologically similar species.1 L/W ratio was calculated from data available on the valve dimensions.

Species	***G. patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov.**	***G. tackei* (Hustedt) Lange-Bert.**	***G. lingulatiformis* (Lange-Bert. & E.Reichardt) Lange-Bert.**	***G. plenkoviciae* G.Udovič & Žutinić**	***G. stoermeri* Kociolek & E.W.Thomas**	***G. tenuis* Levkov & D.M. Williams**
Reference	This study	Hustedt (1942)	Lange-Bertalot (1995)	Udovič *et al.* (2018)	Thomas *et al.* (2009)	Levkov & Williams (2011)
Valve outline	linear-clavate to clavate, with a broader central area in larger specimens	narrow, club-shaped, and slightly expanded transapically in the middle	linear-clavate with undulate margins in larger specimens and only clavate with smooth margins in smaller frustules	narrowly clavate to linear in larger specimens to clavate in smaller specimens, with undulated margins and an inflated centre in longer valves but smaller specimens have convex margins	linear-clavate, with small round granules can be seen by SEM on the valve face; large specimens slightly tumid at the centre	club-shaped, central region tumid
Headpoles	rounded	bluntly rounded	narrowly rounded to nearly acute	rounded and non-protracted	bluntly rounded	obtusely rounded
Footpoles	narrowly rounded	slightly more narrowed than headpoles	narrowly rounded	acutely rounded	narrowly rounded	narrowly rounded
Valve length (µm)	8.0-17.5	10.0-26.0	12.0-50.0	12.0-45.0	10.0-21.0	22.0-42.0
Valve width (µm)	2.5-4.0	*c.* 3	5.5-7.0	3.0-6.5	1.5-3.0	3.5-6.0
L/W ratio	3.4-4.8	–	2.2-7.1	4-6.9	6.7-6.8	6.3-6.8
Valve mantle 1	with a single row of rounded and slit-like areolae	–	–	with two types of areolae: round, 2-4 rows, and in some specimens just single slit-like openings	with a series of round occlusions	clearly high with a single row of round to slit-like areolae
Axial area	rather lanceolate, narrow at the poles and widening towards the central area	narrow, linear	lanceolate and widen near the centre	rather wide, lanceolate to narrow in small specimens	linear, narrow	moderately narrow in smaller specimens and much wider in larger valves
Central area externally	± rhombic, mostly with 2-4 shortened striae, unilateral fascia might be present	small, elongated elliptical to almost circular	almost round	widely elliptical	rectangular	with a broad unilateral fascia
Central nodule internally	slightly raised	–	distinct and rounded	–	small, mound-shaped	slightly raised
Striae	radiate, with single slit-like areolae, becoming almost parallel round poroids near the head- and footpoles	slightly radiate	radiate, becoming parallel near the foot poles, with single areolae and additional 1-2 isolated areolae present	weakly radiate, becoming parallel near the poles, with single areolae, round-shaped at the mid-valve and slightly elongated slits towards the poles, internally areolae longer than externally	parallel to radiate, with single slit-like areolae, 1-3 shortened striae around the central area	radiate to subparallel, with single round areolae near the central area and apices and narrow slit-like areolae at the valve margin, internally areolae elongate and longer than externally
Striae number (in 10 µm)	23-27	28-30, at the middle *c.* 25	13-18	19-22	28-35	14-16
Raphe	straight, filiform	straight, filiform	straight, filiform	straight, filiform	straight, filiform	straight, filiform
External and internal proximal raphe fissures	straight and distinctly dilated into pores, internally T-shaped	–	dilated into pores, internally T-shaped	small, indistinct, internally T-shaped	slightly enlarged, internally with half an anchor (not typically T-shaped) in one direction only	slightly expanded and unilaterally deflected, internally T-shaped
External and internal terminal raphe fissures	with teardrop-shaped pores, internally with relatively large helictoglossae	–	simple and unilaterally deflected, internally with small helictoglossae	short, straight and slightly expanded into drop-like pores, internally with distinct helictoglossae	slightly enlarged, internally with large helictoglossae	slightly expanded to teardrop-like pores, internally with small helictoglossae
Habitats	epilithic	epiphytic in ditches and wet meadows	benthic	in sediments and benthic	epilithic, subaerial	epilithic

from *G. plenkoviciae*. As compared to *G. stoermeri* Kociolek & E.W. Thomas, despite the relative similarity in valve dimensions, there are still several quite sharp differences that could be used to differentiate the two species. For instance, in *G. stoermeri* small round granules can be easily seen using SEM on the valve face, but this feature is totally absent in *G. patrickiana*. *G. stoermeri* also has a much larger L/W ratio than *G. patrickiana*. The axial area in *G. stoermeri* is linear and narrower but it is rather lanceolate in *G. patrickiana*. The central area of *G. stoermeri* most often has a rectangular shape with 1-3 shortened striae, while in *G. patrickiana* it is ± rhombic with 2-4 shortened striae, and in some specimens it even develops into a unilateral fascia. *G. stoermeri* is also characterized by having parallel to radiate striae extending to the raphe slits with much higher density. Also, cingulum bands lack perforations in *G. stoermeri* (LaLiberte 2020). Lastly, the internal proximal raphe endings are completely different: in *G. stoermeri* they have the shape of half an anchor, but they are typically T-shaped in *G. patrickiana* as in the other established species of the genus (Fig. 5C, D). *G. tenuis* Levkov & D.M. Williams also bears some resemblance with *G. patrickiana*. Besides the relatively minor differences in valve outlines, *G. tenuis* can be easily differentiated by having: 1) much larger valve dimensions; 2) lower striation density; 3) striae that externally are most often formed by round areolae but internally are much longer than on the valve exterior; and 4) unilaterally deflected external proximal raphe endings. All these characters make the differentiation between the two species quite simple (Table 2).

Our statistical analyses confirmed that *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov., is much more abundant in the stream with lower nutrient (in particular P) concentrations and higher ecological integrity (and, interestingly, also above-average magnesium concentrations due to the local serpentine geology). Statistical tests also suggest microhabitat and seasonal preferences but also interactions among the three factors (river, habitat, and season) were found to be significant, thus not allowing clear microhabitat or seasonal preferences to be determined. With regard to mineralization and nutrient status, *G. patrickiana* seems to prefer circumneutral to alkaline freshwater habitats with medium-high conductivity and oligo- to mesotrophic conditions. Also, based on the most common and abundant co-occurring diatom species, *G. patrickiana* can be classified as an oligotraphentic/oligo-mesotraphentic species, with moderate tolerance to N enrichment that, however, occurs at the expense of a drastic reduction in abundance and frequency of the species as compared to when it colonizes environments with lower N. Noteworthy is also the observation that *Gomphosphenia patrickiana* Cantonati, Lange-Bert., Kociolek & A.A.Saber, sp. nov., thrives in warm running waters. This is in excellent agreement with the ecological preferences of some *Gomphosphenia* species. In particular, Coste & Ector (2000) recorded the subtropical species *Gomphosphenia oahuensis* in the Adour River, considering it an invasive diatom species in France and an excellent proxy for the warming of running waters in Western Europe due to climate change.

CONCLUSIONS

The combination of characters that identifies *Rhoicosphenula* and separates it from *Gomphosphenia* (pseudosepta present on both poles of the valves, copulae with septa at one of the poles, frustules almost rectangular in girdle view) is so peculiar and straightforward that there would be no doubt in recognizing *Rhoicosphenula* as a separate genus according to current diatom taxonomy standards. A further support of this separation at the genus level comes from the ecological preferences: the only known species of *Rhoicosphenula* (*R. paradoxa* comb. nov.) was collected from a peculiar acidic and dystrophic habitat whilst most *Gomphosphenia* species colonize medium conductivity inland waters. The Neotropical epilithic diatom species *Gomphosphenia patrickiana* is herein proposed as a new species based on a cluster of unique morphological and autecological features. *G. patrickiana* can be easily differentiated from similar species by its valve outline and dimensions, L/W ratio, striation density, and geographical distribution. The most interesting ecological features are the occurrence in warm Neotropical waters and the preference for higher magnesium concentrations.

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